



From waste to feed: Dietary utilisation of bacterial protein from fermentation of agricultural wastes in African catfish (*Clarias gariepinus*) production and health

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ABSTRACT

A feeding trial was conducted to evaluate the effects of replacing fish meal (FM) with bacterial protein (BP) in African catfish (*Clarias gariepinus*) diets. Four iso-nitrogenous (369 g kg⁻¹) and isolipidic (142 g kg⁻¹) diets were formulated to replace graded levels (0%, 30%, 60% and 100%) of FM protein resulting in a dietary inclusion level of 0, 27.90, 55.70 and 93.00 g kg⁻¹ BP. Test diets were fed to the catfish (initial weight 18.17 ± 0.25 g fish⁻¹, n = 20) in three replicate tanks. After 56 days of feeding, catfish fed with 30% BP diet had outperformed (P < 0.05) fish that were fed with the control diet (100% FM) in mean weight gain (MWG), percentage weight gain (PWG), specific growth rate (SGR) and metabolic growth rate (MGR). However, the growth response (MWG, PWG, SGR, and MGR) from diets containing 60% and 100% BP did not differ significantly to the dietary control. The whole-body protein (+8%) and lipid (+12%) contents of the catfish fed 30% BP diet were higher (P < 0.05) than no BP inclusion dietary group. The replacement of FM with BP in the catfish diets did not statistically affect the haematological status (P > 0.05). While histological analysis of the catfish mid-intestine showed intact epithelial barriers with extensive mucosal folds extending into the lumen. The intestinal perimeter ratio of fish fed with 30% BP diet was higher (+32%) than those of the dietary control group (P < 0.05), but not differ from 100% BP-fed diet (P > 0.05). Indication of the liver health status was measured through serum aspartate transaminase, alanine transaminase, and alkaline phosphatase activities, and hepatocyte density, which remains statistically unchanged between the dietary groups (P > 0.05). Overall, the results show that BP can replace FM wholly or partly in African catfish (*C. gariepinus*) diet, without compromising growth performance, haemato-biochemistry, intestinal integrity, or liver functionality.

1. Introduction

The continued use of fish meal (FM) in aquafeeds will not sustainably meet the increasing growth in the aquaculture industry (Hua et al., 2019; Naylor et al., 2009; Troell et al., 2014). Together with increasing cost of FM production and public awareness of the environmental impact of using FM, it is therefore, imperative to develop sustainable alternatives to meet the rising protein demands from the world's fastest-growing food-producing industry (Kim et al., 2019). The question for the strategic use of FM in order to produce better yield of aquatic protein relative to FM was recently addressed by Kok et al. (2020) with emphasis on alternative protein-rich ingredients.

Among the identified alternative protein sources for feed use, microalgae, seaweed, insect meal, and bacterial proteins (BP) have recently gained much research attention (Adeoye et al., 2020a; Fajardo et al., 2020; Fawole et al., 2020; Tlustý et al., 2017; van Raamsdonk et al., 2017; Wan et al., 2019). For the latter, BPs, has shown to be a viable FM replacer that can be produced in large quantities with a potentially low carbon footprint (Jones et al., 2020; Matassa et al., 2016; Pikaar et al., 2017; Spalvins et al., 2018a, 2018b; Spalvins and Blumberga, 2018). This is because BPs can be produced through the fermentation of low-value agricultural waste (e.g. waste from fruit processing, grain processing, sugar production, dairy production, soy protein concentrate production) and industrial biomass residues (e.g.

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waste paper, sulphite waste liquor, lignin waste, latex waste, petroleum residues, Spalvins et al., 2018a, 2018b; Spalvins and Blumberga, 2018). As such, it offers a means to produce a sustainable feed source, which manage and transform waste from other production sectors into a valued commodity (Spalvins et al., 2018a, 2018b).

There is no known potential or report of pathogenic problem of feeding fish with BP. This could be attributed to the utilisation of edible and/or non-pathogenic bacteria such as *Lactobacillus* sp., *Bacillus megaterium*, *Pseudomonas fluorescens*, *Methylococcus capsulatus*, *Rhodococcus opacus* for the production of BP (Mahan et al., 2018; Øverland et al., 2010). In the opinion of Rycken et al. (2017), a BP biomass produced from fermentation with *Escherichia coli* was reported not to pose a safety concern as a feed material though a safe dietary level of 13% was recommended for salmonids. It is important to note that the possible food and feed safety issue could arise with the use of edible BPs, which include contaminations with allergens and toxins (e.g. enterotoxins and cytotoxins) during the production process and potentially toxic metals and persistent organic pollutants introduced from a contaminated feed substrate (Linder, 2019; Ritala et al., 2017). Such contaminations can effectively be controlled and prevented within a closed fermentation system, adherence to industrial verification and validation, food safety production standards (e.g. HACCP systems and ISO standards), and traceability throughout the production cycle.

BPs are known to contain high protein level ranging from 60 to 82% (dry matter), which is similar to soy protein concentrate (48–58%, Heuzé et al., 2020) and fish meal (60–68%, Heuzé et al., 2015). Furthermore, BPs can possess amino acid profiles that are similar to fish meal, while also providing a source of fatty acids, nucleic acids, vitamins and minerals that could support fish growth and normal physiological functions (Bajpai, 2017; Gamboa-Delgado and Márquez-Reyes, 2018; Wang et al., 2020). BPs have been reported to replace FM partly or wholly without negative impact on growth performance or health in farmed finfish species. Previously studied species include farmed rainbow trout (*Oncorhynchus mykiss*, Kiessling and Askbrandt, 1993; Perera et al., 1995a; Hardy et al., 2018), Atlantic salmon (*Salmo salar*, Skrede et al., 1998; Aas et al., 2006; Romarheim et al., 2011; Tlustý et al., 2017), Nile tilapia (*Oreochromis niloticus*, Davies and Wareham, 1988), Florida pompano (*Trachionotus carolinus*, Rhodes et al., 2015), smallmouth grunt (*Haemulon chrysargyreum*, Tlustý et al., 2017), and whiteleg shrimp (*Litopenaeus vannamei*, Tlustý et al., 2017; Hamidoghli et al., 2019). However, there is limited knowledge on how dietary BPs might influence farmed African catfish (*Clarias gariepinus*). This species is a commercially important farmed species for sub-Saharan Africa, Brazil and Asian countries, where the fish provides an affordable high-quality protein food for consumers in the region (FAO, 2020). In 2017, it was reported that the global African catfish (*C. gariepinus*) production was at 230,747 t, with a value of USD > 674 million (FAO, 2019). As such, the present study aims to evaluate the dietary effects of BP on African catfish (*C. gariepinus*) growth performance, body composition, intestinal morphology, haemato-biochemical status, and liver function.

2. Materials and methods

2.1. Diet preparation and experimental design

The test BP (774.80 g kg⁻¹ protein, dry matter) was produced by Yenher Agro-Products Sdn. Bhd. (Malaysia) and obtained from Botson AquaProducts Ltd., Lagos, Nigeria. The BP was produced through bacterial fermentation of agricultural raw material (low-protein plant material, beneath Table 1). Detailed production method of the BP was deemed commercially sensitive and was not shared by the manufacturer. Other ingredients used in the test diets were sourced from a local feed ingredients supplier (ABMN Limited, Ibadan, Nigeria). Four test diets were formulated to be iso-nitrogenous (369 g kg⁻¹), isolipidic (140 g kg⁻¹) and isoenergetic (19 MJ kg⁻¹, Table 1).

The test diets were formulated to have the same feed ingredient

Table 1

Formulation and composition of the experimental diets fed to the African catfish (*Clarias gariepinus*).

Ingredients (g kg ⁻¹)	Diets			
	FM	BP 30%	BP 60%	BP 100%
Fishmeal (72% CP)	100.00	70.00	40.00	0.00
Bacterial protein ^a	0.00	27.90	55.70	93.00
Poultry meal	100.00	100.00	100.00	100.00
Soybean meal (solvent extracted)	350.00	350.00	350.00	350.00
Corn gluten	140.00	140.00	140.00	140.00
Maize meal	210.00	212.10	214.43	217.00
Vegetable oil	79.90	79.90	79.90	79.90
Vitamin & mineral premix ^b	10.00	10.00	10.00	10.00
Antioxidant ^c	0.10	0.10	0.10	0.10
Cassava starch	10.00	10.00	10.00	10.00
Total	1000.00	1000.00	1000.00	1000.00
Proximate composition (g kg ⁻¹)				
Dry matter	915.30	927.00	926.00	907.70
Protein	364.00	366.00	373.40	373.90
Lipid	140.70	147.40	138.10	140.30
Ash	53.00	52.80	51.50	46.90
Nitrogen-free extract	294.90	288.50	290.90	270.60
Crude fibre	62.70	72.30	72.10	76.00
Energy (MJ kg ⁻¹)	19.20	19.40	19.20	19.00

^a Bacterial protein (Yenher Agro-Products Sdn. Bhd., Malaysia): crude protein (77.48%), crude lipid (4.97%), ash (3.58%), crude fibre (1.08%), NFE (8.27%). Amino acid composition (% DM): Aspartic acid (6.01), Threonine (3.05), Glutamic acid (10.00), Glycine (3.80), Alanine (5.30), Valine (3.51), Methionine (2.13), Isoleucine (3.02), Leucine (5.93), Tyrosine (2.30), Phenylalanine (3.15), Lysine (5.14), Histidine (1.55), Arginine (4.25), Proline (3.35), Methionine + Cysteine (2.99), Tryptophan (0.93).

^b Vitamin and mineral premix (per 2.5 kg): 20,000,000 IU vitamin A, 4000,000 IU vitamin D3, 200,000 IU vitamin E, 8000 mg vitamin K3, 20,500 mg vitamin B1, 15,000 mg vitamin B2, 19,500 mg vitamin B6, 15 µg vitamin B12, 90,000 mg Nicotinic Acid, 40,000 mg Pantothenic Acid, 500 mg Folic Acid, 600,000 µg Biotin, 40,000 mg Choline Chloride, 4000 mg Iron, 500 mg Copper, 30,000 mg Manganese, 40,000 mg Zinc, 2000 mg Iodine, 200 µg Selenium, 300,000 mg coated Vitamin C, 50,000 mg Inositol, 750 mg Cobalt, 50,000 mg Lysine, 50,000 mg Methionine and 125,000 mg Antioxidant.

^c Anti-oxidant contains food grade sodium metabisulfite.

composition except for FM being substituted with the BP at 30% (BP 30% diet), 60% (BP 60% diet), and 100% (BP 100% diet). The replacement was on the equivalent protein basis, corresponding to dietary inclusion level of 27.9 g kg⁻¹, 55.7 g kg⁻¹, and 93 g kg⁻¹, respectively (Table 1). As a measure of comparison, a fourth diet was produced without the inclusion of BP. The production of test diets involved homogenising the feed ingredients and subsequently cold extruded (Capsfeed, Ibadan, Nigeria) to produce 2 mm diameter pellets. The diets were oven-dried at 60 °C for 12 h. The dried diets were stored in airtight containers before use.

The feeding trial was conducted at the Department of Aquaculture and Fisheries Management of the Federal University of Agriculture, Abeokuta, Nigeria. African catfish (*Clarias gariepinus*) were sourced from a reputable local fish hatchery (Motherhood Fish Farm, Abeokuta, Nigeria) and randomly distributed into twelve 50 L rectangular plastic tanks. The catfish were acclimatised for two weeks prior to the start of the feeding trial. The fish tank containing 20 catfish fingerlings (18.17 ± 0.25 g fish⁻¹) each were randomly assigned to each dietary treatment. The catfish were fed the respective experimental diet twice daily (0800 h and 1700 h) to apparent satiation for 56 days. The photoperiod (~17 h: 7 h, light: dark) and water temperature (29 ± 0.4 °C) were maintained at ambient condition. Water quality parameters were monitored weekly: pH, 6.93 ± 0.53 (HI98107 pHep®, Hanna Instruments, Leighton Buzzard, UK), dissolved oxygen, > 5 mg L⁻¹ (HI3810, Hanna Instruments, Leighton Buzzard, UK), and ammonia nitrogen, 0.23 ± 0.1 mg L⁻¹ (HI3824, Hanna Instruments, Leighton Buzzard, UK).

2.2. Growth, feed efficiency, somatic indices and tissue sampling

On termination of the feeding trial, the African catfish were starved for 24 h and batch weighed. Growth performance, feed efficiency and somatic indices of the catfish fed the experimental diets were assessed using mean weight gain (MWG), specific growth rate (SGR), metabolic growth rate (MGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV), lipid productive value (LPP), hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor (K). Calculations were carried out using the following formulae.

Feed Intake, **FI** = Total feed consumed (g)/Number of fish harvested.

Specific Growth Rate, **SGR** = $((\ln \text{FBW} - \ln \text{IBW})/T) \times 100$.

where FBW = final body weight (g), IBW = initial body weight (g), and T = time in days

Metabolic Growth Rate, **MGR**

$$= (\text{Net weight gain in g}) / \{[(\text{IBW}/1000)^{0.8} + (\text{FBW}/1000)^{0.8}] / 2\} / \text{feeding duration in days}$$

where FBW = final body weight (g) and IBW = initial body weight (g)

Feed Conversion Ratio, **FCR** = FI/WG

where FI = feed intake (g) and WG = wet weight gain (g)

Protein Efficiency Ratio, **PER** = WG/PI

where WG = wet weight gain (g) and PI = protein ingested (g)

Protein Productive Value, **PPV**

$$= 100 \times [(\text{FBW} \times \text{FBPC}) - (\text{IBW} \times \text{IBPC})] / (\text{PCD} \times \text{TFI})$$

where FBW = final body weight (g), FBPC = final body protein content (g), IBW = initial body weight (g), IBPC = initial body protein content (g), PCD = protein content in diet (g) and TFI = total feed intake (g)

Lipid Productive Value, **LPV**

$$= 100 \times [(\text{FBW} \times \text{FBLC}) - (\text{IBW} \times \text{IBLC})] / (\text{LCD} \times \text{TFI})$$

where FBW = final body weight (g), FBLC = final body lipid content (g), IBW = initial body weight (g), IBLC = initial body lipid content (g), LCD = lipid content in diet (g) and TFI = total feed intake (g)

Hepatosomatic Index, **HSI** = (LW/BW) $\times$ 100

where LW = liver weight (g) and BW = body weight (g)

Viscerosomatic Index, **VSI** = (VW/BW) $\times$ 100

where VW = visceral weight (g) and BW = body weight (g)

Condition Factor, **K** = $(100 \times (\text{BW}) / \text{TL}^3)$

where BW = body weight (g) and TL = total length (cm)

All tissue collection was carried out after the fish were humanely euthanised using clove oil at a concentration of 100 mg L⁻¹ followed by the destruction of the brain.

2.3. Haemato-biochemical parameters

At the end of the feeding trial, two fish per tank ($n = 6$) were randomly selected and anaesthetised with 100 mg L⁻¹ clove oil. Blood was collected from the caudal arch of the fish using a 25-gauge needle and 1 mL syringe. Subsequently, the blood was transferred into heparinised tube for basic haematological analysis. The haemoglobin, red blood cell count, white blood cell count, and differential leucocyte count were determined according to standard methods (Lewis et al., 2006). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as described in Haney et al. (1992) using the

following formulae:

Mean corpuscular volume, **MCV**

$$= \text{haematocrit (\%PCV)} \times 10 / \text{RBC (x10}^9 \text{ L}^{-1}\text{)};$$

Mean corpuscular haemoglobin, **MCH**

$$= (\text{haemoglobin (g dL}^{-1}\text{)} - 1) \times 10 / \text{RBC (x10}^{12} \text{ L}^{-1}\text{)};$$

Mean corpuscular haemoglobin concentration, **MCHC**

$$= (\text{haemoglobin (g dL}^{-1}\text{)} \times 100) / \text{haematocrit (\%PCV)}.$$

Blood was collected from another two fish in each tank ($n = 6$) without the use of an anticoagulant. Samples were subsequently centrifuged at 3000 $\times$ g for 10 min at 4 °C and the collected serum was stored in -20 °C for biochemical analysis. The serum samples were used to measure the activity of serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) using commercial kits (Randox Laboratories Limited, Crumlin, United Kingdom).

2.4. Proximate composition

At the end of the feeding trial, two fish were sampled from each tank ($n = 6$) for proximate analysis. Proximate analysis of the diets and the fish body composition was carried out using AOAC (2005) standard methods. Briefly, dry matter was measured through drying samples at 105 °C for 48 h until a constant weight was achieved. Crude protein and lipid contents were determined using Kjeldahl method and Soxhlet ether method, respectively. The ash content was determined by incinerating the sample in muffle furnace at 550 °C for 8 h. The samples crude fibre was determined by fibretherm digestion and filtration method. Nitrogen-free extract (NFE) and energy (MJ/kg) values were calculated using the following formulae:

$$\text{NFE} = \text{Dry matter, \%} - (\text{Crude protein, \%} + \text{Crude lipid, \%} + \text{Ash, \%} + \text{Crude fibre, \%})$$

Energy (MJ/kg)

$$= (\text{Crude protein, \%} \times 23.6) + (\text{Lipid, \%} \times 39.5) + (\text{NFE, \%} \times 17).$$

2.5. Intestinal and liver histology

At the conclusion of the feeding trial, two fish per tank ($n = 6$) were sampled for mid-intestine and liver histological examination. The samples were fixed in 10% neutral buffered formalin, processed in graded ethanol and xylene. The processed tissues were embedded in paraffin wax, sectioned (5 μ m thickness) and stained with Periodic acid-Schiff (mid-intestine) or haematoxylin and eosin (liver). The processed histological slides were imaged using a light microscope (BX53, Olympus Life Science, Tokyo, Japan) and morphological measurements were carried out using ImageJ (version 1.51, National Institute of Health, Bethesda, Maryland, USA). The mid-intestinal perimeter ratio (PR), the number of intraepithelial leucocytes (IELs) and goblet cells abundance in the epithelium were assessed and calculated as previously described in Adeoye et al. (2020b). From the liver images, the total number of cell nuclei was counted in five randomly selected non-overlapping fields-of-view (Wang et al., 2019).

2.6. Statistical analysis

All data are presented as mean values with its corresponding standard deviation. Normality and equality of variance of the data were checked using Kolmogorov-Smirnov test and Bartlett's test, respectively. Data were analysed using one-way analysis of variance (ANOVA) followed by a post-hoc Duncan multiple range test. Differences were

Table 2Growth performance, nutrient utilisation and somatic indices of African catfish (*Clarias gariepinus*) after being fed with test diets for 56 days ($n = 3$, $\pm$ SD).

	Diets				P-value
	FM	BP 30%	BP 60%	BP 100%	
IBW (g fish ⁻¹)	18.17 $\pm$ 0.29	18.33 $\pm$ 0.29	18.17 $\pm$ 0.29	18.00 $\pm$ 0.00	0.487
Feed intake (g fish ⁻¹)	81.51 $\pm$ 4.45 ^a	92.08 $\pm$ 6.10 ^b	75.06 $\pm$ 2.28 ^a	81.03 $\pm$ 4.43 ^a	0.032
MWG (g fish ⁻¹)	52.51 $\pm$ 2.34 ^a	63.16 $\pm$ 3.76 ^b	49.24 $\pm$ 2.46 ^a	50.95 $\pm$ 3.06 ^a	0.006
PWG (%)	373.83 $\pm$ 9.08 ^a	407.29 $\pm$ 12.71 ^b	354.85 $\pm$ 18.97 ^a	351.29 $\pm$ 16.13 ^a	0.006
SGR (% day ⁻¹)	2.40 $\pm$ 0.04 ^a	2.55 $\pm$ 0.05 ^b	2.30 $\pm$ 0.08 ^a	2.28 $\pm$ 0.07 ^a	0.007
MGR (g kg ^{-0.8} day ⁻¹)	11.56 $\pm$ 0.18 ^a	12.29 $\pm$ 0.20 ^b	11.06 $\pm$ 0.37 ^a	10.96 $\pm$ 0.33 ^a	0.006
FCR	1.55 $\pm$ 0.06	1.46 $\pm$ 0.14	1.52 $\pm$ 0.08	1.59 $\pm$ 0.03	0.549
PER	1.39 $\pm$ 0.05 ^a	1.42 $\pm$ 0.07 ^a	1.18 $\pm$ 0.09 ^b	1.22 $\pm$ 0.03 ^b	0.230
PPV	20.22 $\pm$ 1.94	21.32 $\pm$ 0.43	17.63 $\pm$ 0.72	19.75 $\pm$ 3.03	0.178
LPV	47.71 $\pm$ 5.01	50.72 $\pm$ 1.66	48.44 $\pm$ 3.59	50.96 $\pm$ 5.63	0.732
K	0.71 $\pm$ 0.03	0.76 $\pm$ 0.10	0.73 $\pm$ 0.02	0.73 $\pm$ 0.11	0.917
HSI	1.29 $\pm$ 0.05	1.32 $\pm$ 0.03	1.42 $\pm$ 0.32	1.49 $\pm$ 0.14	0.524
VSI	10.51 $\pm$ 1.08	11.49 $\pm$ 1.02	10.18 $\pm$ 0.28	10.35 $\pm$ 0.26	0.235
Survival (%)	86.67 $\pm$ 12.58	88.33 $\pm$ 2.89	95.00 $\pm$ 5.00	88.33 $\pm$ 10.41	0.664

Values with different superscripts on the same row indicates there is a significant difference ($P < 0.05$). Abbreviations: IBW, initial mean body weight; FBW, final mean body weight; MWG, mean weight gain; PWG, percentage weight gain; SGR, specific growth rate; MGR, metabolic growth rate; FCR, feed conversion ratio; PER, protein efficient ratio; PPV, protein productive value; LPV, lipid productive value; K, condition factor; HSI, hepatosomatic index; VSI, viscerosomatic index.

considered significant when $P < 0.05$.

3. Results

3.1. Growth performance, nutrient utilisation, and somatic indices

After 56 days of feeding African catfish with bacterial protein, growth performance, nutrient utilisation and somatic indices were assessed through MWG, SGR, MGR, FCR, PER, PPV, LPV, K, HSI and VSI (Table 2). The experimental fish increased in weight by up to four-fold. Catfish that were fed with BP 30% diet had significantly increased feed intake (+13%), MWG (+20%), PWG (+9%), SGR (+6%) and MGR (+6%) when compared with the control group (FM, $P < 0.05$). While, the growth performance indices (MWG, PWG, SGR and MGR) were unaffected when the fish were fed up to 100% BP compared with those fed FM (control) diet ($P > 0.05$). The replacement of FM at any level with BP did not have a significant impact on the nutrient utilisation (FCR, PER, PPV and LPV, $P > 0.05$), somatic indices (K, HSI and VSI, $P > 0.05$), or survival of the fish. Although FI was significantly higher among the fish fed with BP 30% diet compared to other dietary groups ($P < 0.05$), nonetheless all the fish were observed to readily accept the experimental diets even at 100% BP inclusion, (FI = 75.06–92.08 g fish⁻¹).

3.2. Whole-body composition

The whole-body composition of the African catfish fed with the test diets is presented in Table 3. The result shows that the body protein content (+8%) was significantly higher in BP 30% dietary group than those fed the control FM diet ($P < 0.001$). The lowest protein content was however found in fish that were fed with BP 60% diet in contrast to the control ($P < 0.001$). All fish fed with BP diets had significantly

higher body lipid content (+12% in BP 30%, +9% in BP 60%, and +11% in BP 100%) than the fish receiving the dietary control ($P < 0.001$). For body ash content, the levels were significantly higher in BP 100% fed fish than any other treatments ($P < 0.001$).

3.3. Haemato-biochemical parameters

Analysis from the blood collected at the end of the feeding trial showed that there were no differences in the measured haematological parameters (PCV, haemoglobin, red blood cells count, white blood cells count, and differential leucocyte, MCV, MCH and MCHC) between the dietary treatments ($P > 0.05$, Table 4). The serum AST, ALT and ALP showed no significant differences among the various treatment groups ($P > 0.05$, Table 4).

3.4. Intestinal and liver histology

Histological analysis indicated that all dietary catfish group mid-intestine epithelial barriers were intact with extensive mucosal folds into the lumen (Fig. 1). Each mucosal fold consisted of simple lamina propria with abundant intraepithelial leucocytes (IELs) and mucous-secreting goblet cells. There was no significant difference in the mucosal fold width, goblet cells or IELs abundance between the different dietary fish groups ($P > 0.05$, Table 5). Although, the mean mucosal fold heights were higher as dietary BP inclusion was increased, but this trend was found not to be statistically significant ($P > 0.05$). In comparison, the mid-intestinal perimeter ratio was significantly increased (+32%) in fish fed with BP 30% diet when compared to the control (FM diet, $P < 0.05$). All fish sampled for liver histological analysis had shown that the hepatic cellular structure arrangement was regular with clear boundaries (Fig. 2). There was a general trend of growing mean total number of liver nuclei as dietary BP inclusion were

Table 3Whole-body composition of African catfish (*Clarias gariepinus*) after being fed with test diets for 56 days ($n = 3$, $\pm$ SD, % wet weight basis).

	Diets				P-value
	FM	BP 30%	BP 60%	BP 100%	
Moisture	74.73 $\pm$ 1.34	72.48 $\pm$ 0.00	74.50 $\pm$ 3.03	72.67 $\pm$ 2.47	0.078
Crude protein	12.81 $\pm$ 0.16 ^a	13.79 $\pm$ 0.13 ^c	12.15 $\pm$ 0.10 ^b	12.81 $\pm$ 0.08 ^a	< 0.001
Crude lipid	8.66 $\pm$ 0.11 ^a	9.73 $\pm$ 0.13 ^b	9.42 $\pm$ 0.10 ^c	9.59 $\pm$ 0.02 ^c	< 0.001
Ash	2.13 $\pm$ 0.05 ^{ab}	2.04 $\pm$ 0.07 ^a	2.27 $\pm$ 0.04 ^b	2.60 $\pm$ 0.08 ^c	< 0.001

Values with different superscripts on the same row indicates there is a significant difference ($P < 0.05$).

Table 4Haemato-biochemical parameters of African catfish (*Clarias gariepinus*) after being fed with test diets for 56 days (n = 3, $\pm$ SD).

	Diets				P-value
	FM	BP 30%	BP 60%	BP 100%	
PCV (%)	29.67 $\pm$ 5.25	33.17 $\pm$ 4.25	32.17 $\pm$ 1.15	33.67 $\pm$ 5.03	0.677
Haemoglobin (g dL ⁻¹)	10.02 $\pm$ 1.63	11.28 $\pm$ 1.43	10.78 $\pm$ 0.42	11.38 $\pm$ 1.81	0.647
RBC (cells $\times 10^{12}$ L ⁻¹)	1.86 $\pm$ 0.32	2.36 $\pm$ 0.56	2.19 $\pm$ 0.19	2.18 $\pm$ 0.56	0.597
WBC (cells $\times 10^9$ L ⁻¹)	137.00 $\pm$ 35.17	140.92 $\pm$ 23.46	198.67 $\pm$ 78.12	147.37 $\pm$ 50.65	0.461
Neutrophil (%)	20.67 $\pm$ 2.93	19.00 $\pm$ 5.77	33.00 $\pm$ 14.65	24.33 $\pm$ 6.00	0.265
Lymphocytes (%)	77.00 $\pm$ 3.04	76.00 $\pm$ 7.76	62.83 $\pm$ 16.85	69.33 $\pm$ 3.21	0.304
Basophil (%)	0.50 $\pm$ 0.50	0.17 $\pm$ 0.29	0.50 $\pm$ 0.50	0.17 $\pm$ 0.29	0.596
Eosinophil (%)	0.33 $\pm$ 0.29	2.50 $\pm$ 2.00	2.33 $\pm$ 1.44	2.50 $\pm$ 1.32	0.244
Monocytes (%)	1.50 $\pm$ 0.50	2.33 $\pm$ 2.02	1.33 $\pm$ 0.76	0.67 $\pm$ 0.29	0.394
MCV (fL)	159.87 $\pm$ 17.23	144.53 $\pm$ 18.38	149.22 $\pm$ 7.05	160.68 $\pm$ 22.71	0.612
MCH (pg)	53.60 $\pm$ 5.52	49.00 $\pm$ 5.81	50.02 $\pm$ 2.64	61.72 $\pm$ 11.84	0.210
MCHC (g dL ⁻¹)	33.88 $\pm$ 0.71	33.81 $\pm$ 0.38	33.53 $\pm$ 0.28	33.82 $\pm$ 0.37	0.799
AST (IU L ⁻¹)	117.87 $\pm$ 8.81	153.68 $\pm$ 3.43	154.43 $\pm$ 34.33	178.87 $\pm$ 89.20	0.518
ALT (IU L ⁻¹)	21.48 $\pm$ 2.13	27.35 $\pm$ 12.56	19.53 $\pm$ 1.82	20.23 $\pm$ 5.51	0.539
ALP (IU L ⁻¹)	56.38 $\pm$ 1.72	69.12 $\pm$ 19.75	77.87 $\pm$ 11.83	54.05 $\pm$ 4.43	0.115

There were no significant differences ($P > 0.05$) across the treatments except in ALP. PCV, packed cell volume; RBC, red blood cells; WBC, leucocytes; %, mean percentage of total leucocytes; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; AST, aspartate transaminase (IU L⁻¹); ALT, alanine transaminase (IU L⁻¹); ALP, alkaline phosphatase (IU L⁻¹).

increased, however, statistical analysis revealed this was not significant ($P > 0.05$, Table 5).

4. Discussion

To the authors' knowledge, the present study is the first-ever

reported feeding trial that tests the viability of BP as a FM replacer in African catfish (*C. gariepinus*) formulated diet. BPs have previously been reported to contain high protein content (~60–82%, dry matter) which is higher and more digestible than other alternative protein sources e.g. microalgae, seaweed, insect meal (Gamboa-Delgado and Márquez-Reyes, 2018; Jones et al., 2020; Spalvins and Blumberga, 2018; Wang

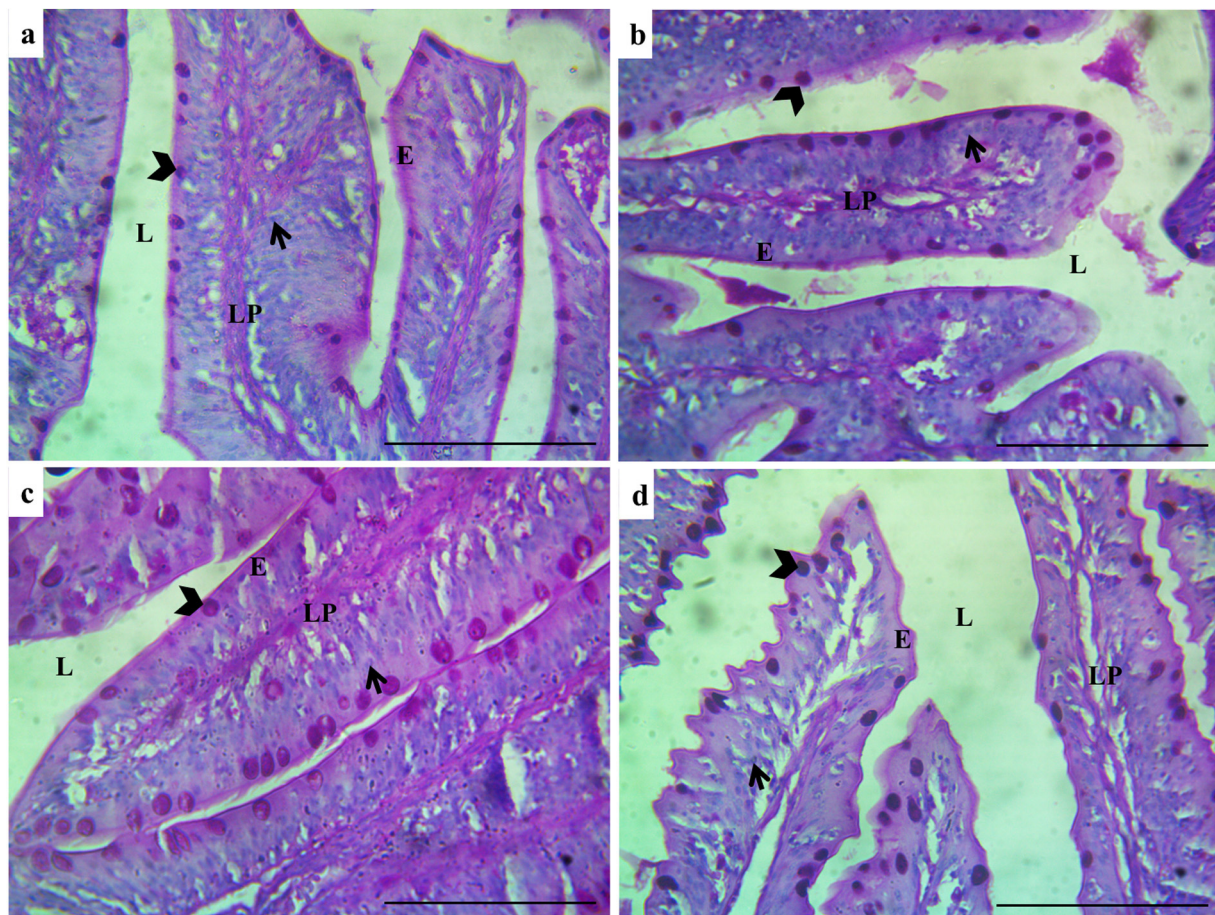


Fig. 1. The mid-intestine of African catfish (*Clarias gariepinus*) fed with FM (a), BP 30% (b), BP 60% (c) and BP 100% (d) diets after 56 days. Goblet cells (arrows) and abundant IELs (arrowheads) are present in the epithelia. Abbreviations are E enterocytes, LP lamina propria and L lumen. Stained with Periodic Acid Schiff (Scale bar = 100 μ m).

Table 5Intestinal and liver histology of African catfish (*Clarias gariepinus*) after being fed with test diets for 56 days (n = 3, $\pm$ SD).

	Diets				P-value
	FM	BP 30%	BP 60%	BP 100%	
Mucosal fold height (μ m)	764.02 $\pm$ 66.83	829.01 $\pm$ 19.66	719.42 $\pm$ 100.53	778.00 $\pm$ 92.31	0.581
Mucosal fold width (μ m)	135.18 $\pm$ 13.19	133.67 $\pm$ 9.36	137.40 $\pm$ 12.48	120.93 $\pm$ 6.94	0.465
Perimeter ratio (AU)	2.90 $\pm$ 0.25 ^a	3.83 $\pm$ 0.33 ^b	2.85 $\pm$ 0.27 ^a	3.68 $\pm$ 0.10 ^b	0.006
Goblet cells (per 100 μ m)	4.55 $\pm$ 1.13	4.33 $\pm$ 1.12	4.06 $\pm$ 1.03	4.28 $\pm$ 1.15	0.296
IELs (per 100 μ m)	45.18 $\pm$ 11.99	50.32 $\pm$ 16.64	43.97 $\pm$ 14.83	47.34 $\pm$ 13.53	0.187
Liver nuclei (No/ FV)	261.92 $\pm$ 68.39	268.92 $\pm$ 72.59	273.92 $\pm$ 72.71	301.17 $\pm$ 68.46	0.551

There were no significant differences ($P > 0.05$) across the treatments except in the intestinal perimeter ratio. AU, arbitrary units and IELs, Intraepithelial leucocytes. Liver nuclei (No/ FV) is the total number of liver nuclei (per field of view under $40\times$ objective magnification).

et al., 2020). In addition to the protein content, BP also contains other nutrients such as fatty acids, nucleic acids, vitamins and minerals (Gamboa-Delgado and Márquez-Reyes, 2018). The production of BP from waste streams such as agricultural or industrial by-products further offers the benefit in reducing environmental pollution and potentially be produced in a low-cost manner that would be competitive against FM (Matassa et al., 2016; Pikaar et al., 2017; Spalvins et al., 2018a, 2018b; Spalvins et al., 2018a; Wang et al., 2020).

In the present study, African catfish (*C. gariepinus*) were fed with diets that had increasing inclusion level of BP at the expense of FM (0, 30, 60, and 100%). After 56 days of feeding, catfish fed with BP 30% diet had higher growth performance than those fed with no BP inclusion (100% FM) diet. Similarly, improved performances were observed in Nile tilapia (*O. niloticus*, +30% weight gain and +30% SGR) and

Malaysian mahseer (*Tor tambroides*, +19.7% weight gain and +16.7% SGR) when fed with diets containing 50% BP from *Rhodovulum sulfidophilum* and *Marichromatium sp.*, respectively (Banerjee et al., 2000; Chowdhury et al., 2016). The observed growth performance could be attributed to a positive synergistic effect of combining two high biological value ingredients (complimentary proteins) in the diet i.e. FM and BP. In addition to the high nutritional value, the presence of enzymes in the BP for early digestion and enhanced metabolism could be responsible for improved growth performance of the fish (Chowdhury et al., 2016). The improved fish performance could also be due to the bacterium in BP being able to synthesize precursors of cell macromolecules and vitamins (Manju and Dhevendran, 1997). Furthermore, the enhanced growth response observed in the present study at the lower (30% BP) inclusion level may also be explained by the higher

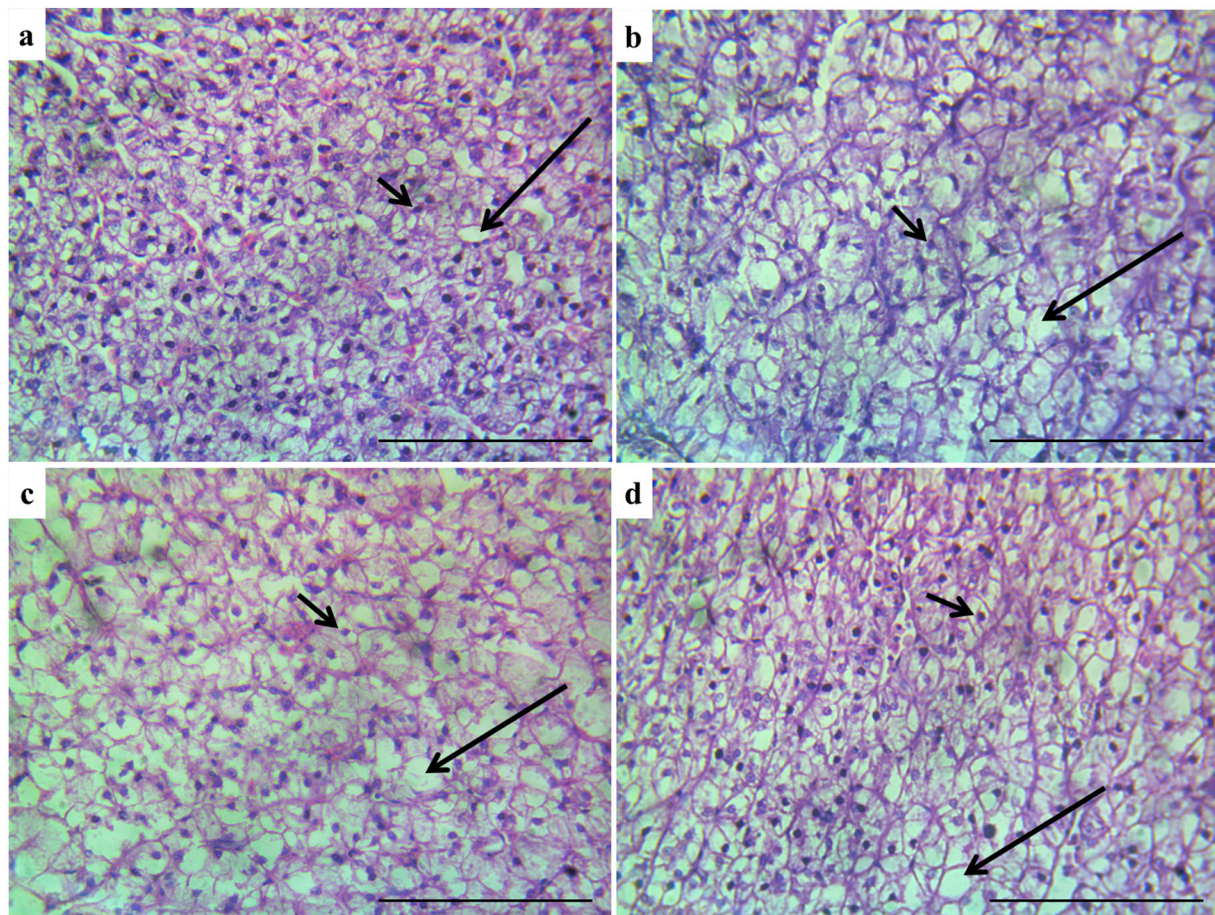


Fig. 2. The liver structure of African catfish (*Clarias gariepinus*) fed with FM (a), BP 30% (b), BP 60% (c) and BP 100% (d) diets after 56 days. The arrows indicate a hepatic cell and their nuclei (short arrow) showing progressive lipid vacuolisation (long arrow) compared to the FM fed group. Stained with Haematoxylin and Eosin (Scale bar = 100 μ m).

feed intake observed in this treatment as the nutrient utilisation among the dietary groups were equal. The unchanged level of nutrient utilisation (FCR, PER, PPV, LPV) observed between the dietary groups signify that the addition of BP up to 100% would not impair the nutritional quality of African catfish (*C. gariepinus*) diet.

The growth response in the catfish fed with BP 60% or 100% diets showed no apparent differences to the control fish group. This unchanged growth response in the current study is comparable with the observations of [Tlustý et al. \(2017\)](#). There was no significant change in growth performance and nutrient utilisation of shrimp (*L. vannamei*, 100% FM replacement), smallmouth grunt (*H. chrysargyreum*, 30% FM replacement) and Atlantic salmon (*S. salar*, 55% FM replacement) when fed with BP (*Methylobacterium extorquens*) inclusion diets ([Tlustý et al., 2017](#)). In another vein, a reduction in growth performance (−36% weight gain and −18% SGR) was reported in Atlantic salmon (*S. salar*) when BP (from a mix of *Methylococcus capsulatus*, (Bath), *Alcaligenes acidovorans*, *Bacillus brevis*, and *Bacillus firmus*) was used to replace 50% FM amino acids in diet ([Storebakken et al., 2004](#)). Poor growth performance was also observed in rainbow trout (*O. mykiss*) when fed 100% BP diet ([Perera et al., 1995b](#)). Similarly, [Newport and Keal \(1980\)](#) reported reduced growth performance in swine when fed with BP (at 60 or 80% milk protein replacement). The possible imbalance in the amino acid composition of BP and low digestibility due to bacterial cell walls and membranes resistant to enzymatic digestion could be responsible for the poor performance at relatively high BP inclusion level ([Kiesling and Askbrandt, 1993](#)). In addition, increase in dietary inclusion of BP has also been linked to reduction in nitrogen digestibility, low amino acids absorption efficiency and subsequently low total digestible amino acids ([Perera et al., 1995b](#); [Storebakken et al., 2004](#)). Hence, feeding fish with diet high in BP could lead to limiting dietary amino acids supply for growth.

There was no observable palatability-mediated diet rejection in the present study. The similar FIs noted in the catfish fed the control, BP 60% and BP 100% diets indicated that the catfish responded well to the diets even though significantly lower to FI in BP 30% dietary group. [Hardy et al. \(2018\)](#) reported a reduction in FI when rainbow trout (*O. mykiss*) was fed with diet containing BP (*M. extorquens*) at 10% soybean replacement. In another study, high inclusion of BP (62.5 or 100%) in rainbow trout (*O. mykiss*) diet resulted in higher feed consumption but reduced nitrogen absorption efficiency, increased urea excretion and consequently reduced growth rates ([Perera et al., 1995a](#)). However, the inclusion of BP (at 10 or 20% FM replacement) in Atlantic salmon (*Salmo salar*) diet raised in saltwater did not have significant effect on the fish FI and growth ([Marit Berge et al., 2005](#)).

Fish mortality can be caused by poor water quality, feed deprivation, potentially toxic compounds in the feed (e.g. mycotoxins, anti-nutrients and toxic metals), and stress from handling. However, in the current study, the survival (86.67–95.00%) among the dietary groups was relatively high and was not significantly different. Similarly, [Delamare-Deboutteville et al. \(2019\)](#) reported that feeding a series of diets containing BP (at 33, 66 or 100% FM replacement) to Asian sea bass (*Lates calcarifer*) did not significantly affect the fish survival. Improved survivals were however reported in swine (+27.3% when fed 60 or 80% BP diet, [Newport and Keal, 1980](#)) and Nile tilapia (+21% when fed 50% BP, [Banerjee et al., 2000](#)). On the contrary, lower survival (−1.6%) was observed in Atlantic salmon (*S. salar*) when fed BP (at 50% FM replacement, [Storebakken et al., 2004](#)).

The whole-body composition is a measure of diet utilisation by fish and a nutritive value, which will have an economic impact on the final product and consumer acceptance. In this study, the catfish fed BP 30% diet had the highest measured body protein content than the group with no inclusion of BP. [Chowdhury et al. \(2016\)](#) also reported improvement in protein (+9.3%), ash (+27.8%) and lipid (+45.2%) body contents of Malaysian Mahseer (*T. tambroides*). According to [Shearer \(1994\)](#), variations in body composition of farmed fish could be caused by factors such as genetic (fish size, sex and stage of growth), environmental/

water quality parameters, and dietary (diet composition, feeding frequency, ration level, etc.) influences. The positive synergistic effect of FM and replacing 30% of FM with BP in the diet resulted in superior fish growth and nutrient absorption. This has subsequently given a high protein retention and protein productive value in the present investigation. On the contrary, [Newport and Keal \(1980\)](#) presumed that nucleic acids in BP are metabolised but not retained for tissue synthesis, and concluded that dietary BP cannot influence carcass protein in swine.

The whole-body lipid and ash (in BP 100% group) contents of the catfish fed BP diets were significantly higher than those fed the control diet. The observed increase in the body lipid content of the fish that were fed with BP inclusion diets could be associated with the retention of lipids in the body. Similar results were reported in whiteleg shrimp (*L. vannamei*, [Hamidoghli et al., 2019](#)), Atlantic salmon (*S. salar*, [Storebakken et al., 2004](#)), and rainbow trout (*O. mykiss*, [Hardy et al., 2018](#); [Hauptman et al., 2014](#)). [Hauptman et al. \(2014\)](#) and [Hamidoghli et al. \(2019\)](#) suggested that the higher whole-body lipid contents observed at higher inclusion of the BPs could be due to an attempt by the fish to compensate for the imbalance in BP amino acids. This has resulted in an increase of protein deamination into ammonia. The ammonia waste is then eliminated from the body and the non-nitrogenous or carbonaceous component of the diet deposited as lipid, and this might be the reason for the increased whole-body lipid content noticed in this study. Dietary nucleotides (which may be increased with the inclusion levels of BP in the catfish diets) have been reported to affect gut motility, thereby increasing digesta transit time and subsequent promotion of an increased mineral uptake ([Kim et al., 1968](#)). This may be responsible for the high body ash content of the catfish fed BP 100% diet.

Measurement of haematological parameters are important tools for assessing the fish health and physiological wellbeing when novel ingredients are evaluated in aquafeeds ([NRC, 2011](#)). In this study, the measured parameters such as packed cell volume, haemoglobin, red blood cell count, white blood cell count, and differential leukocyte count, did not show any apparent changes when catfish were fed with the BP inclusion diets. [Marit Berge et al. \(2005\)](#) also observed that the inclusion of BP in Atlantic salmon (*S. salar*) diet did not significantly alter the haematocrit value of the fish. These observations suggest that feeding BP to African catfish (*C. gariepinus*) would not elicit any adverse effect on the fish physiological function and health. Although, the WBC results were significantly the same among the dietary groups, however, the average WBC values are higher in BP groups. This may signify a beneficial immune response from dietary BP.

The intestinal health of the catfish fed with BP was also assessed through histological examination using indicators such as mucosal fold height, mucosal fold width, mid-intestinal perimeter ratio, the abundance of goblet cells and IELs. Catfish fed with 30% BP diet yielded a higher mid-intestinal perimeter ratio (a measure of nutrient absorption and utilisation) when compared to those fed the control diet (100% FM) diet. Similarly, the inclusion of BP in diet of Atlantic salmon (*S. salar*) raised in both freshwater and saltwater conditions did not negatively affect the morphology of the fish intestine ([Marit Berge et al., 2005](#); [Storebakken et al., 2004](#)). The improvement in the intestinal perimeter ratio of fish in the present study could be due to complementary changes to meet the increased rates of digestion and absorption of nutrients after exposure to the superior quality (30% BP) diet. The improved nutrient absorption and utilisation observed in the catfish fed 30% BP diet eventually translated to improved growth performance when compared with those of the catfish fed other diets. However, there was no significant difference in the mid-intestinal perimeter ratios of the catfish fed 30% and 100% BP diets.

The leakage of AST, ALT and ALP enzymes into the blood could be indicative of liver damage and/or bile duct obstruction ([Fawole et al., 2020](#)). As such, an increase in the liver-related enzyme activities in the blood implies a deterioration in the liver function. [Amer et al. \(2019\)](#)

reported a significant increase in the serum levels of AST and ALT enzymes in Nile tilapia (*O. niloticus*) fed whey protein concentrate and this led to a decline in the liver function. In the present study, there was no apparent effect of the dietary BP on the catfish liver functionality, which was determined by serum AST, ALT, and ALP levels. Similarly, there appears to be an increase in the total number of liver nuclei as the inclusion level of the BP increases in the catfish diet, but this was found not statistically different. Storebakken et al. (2004) and Marit Berge et al. (2005) observed well differentiated structure of hepatic tissue with no sign of pathological condition when Atlantic salmon (*S. salar*) was fed BP diet. The dietary exogenous nucleotides as found in the BP can play a significant role in the repair and regeneration of liver damage, as deprivation of nucleotides would reduce the hepatic protein synthesis rate as shown in the cirrhotic rat model (Pérez et al., 2004). However, a future study may test if BP nucleotides can be assimilated by hepatocytes and raise protein synthesis, leading to activation of AST, ALT and ALP. Overall, it would be interesting to determine whether BP is responsible for liver-associated enzymes leaking into the catfish circulatory system, but not necessarily due to liver impairment.

5. Conclusion

It can be concluded from this study that FM can be wholly or partly replaced with BP in African catfish (*C. gariepinus*) formulated diet without any negative impacts on growth performance, haemato-biochemistry, intestinal histology and liver functionality. Furthermore, the replacement of 30% FM with BP had resulted in higher growth performance compared to other dietary groups. This suggests that 30% replacement with BP is the most appropriate level for farmed African catfish (*C. gariepinus*). The use of such secondary proteins with a high biological value derived from waste streams of other production sectors will offer a sustainable solution for farmed African catfish expansion. BP will serve to mitigate FM dependency and carbon footprint, whilst being an economically competitive feed ingredient.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the study reported in this paper.

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